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Collaborative Effort to Model Plant Response to Acidic Rain. Journal Article

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16. ABSTRACT

Radish plants were exposed three time per week to simulated acidic rain at pH values of 2.6 to 5.4 over the course of four weeks in trials performed at Argonne, IL: Ithaca & Upton, NY; Corvallis, OR; Oak Ridge, TN; and Toronto, Canada. Uniform genotype, soil media and planting techniques, treatment procedures, biological measurements, and experimental design were employed. Growth of plants differed among trials as a result of variation in greenhouse environmental conditions according to location and facilities. Larger plants underwent greater absolute but lower relative reduction in biomass after exposure to the high levels of acidity. A generalized Mitscherlich function was used to model the effects of acidity of simulated rain or dry mass of hypocotyls using data from three laboratories that performed only one trial each, were used to test the model. When the laboratory by trial effect was removed (influence of different growth conditions), lack of fit to the Mitscherlich function was insignificant. Thus, a single mathematical model satisfactorily characterized the relationship between acidity and mean plant response. The pH value associated with a 10 percent reduction in mass was 3.3+ 0.3 for hypocotyls. No value was estimated for shoots because effects on shoots were not significant. The results of this study demonstrate that a generalized exposure-response model can be developed in the presence of large variations in environmental conditions when plant culture and exposure to simulated rain are standardized among laboratories.

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A Collaborative Effort to Model Plant Response to Acidic Rain

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Radish plants were exposed three times per week to simulated acidic rain at pH values of 2.6 to 5.4 over the course of four weeks in trials performed at Argonne, Illinois; Ithaca and Upton, New York; Corvallis, Oregon; Oak Ridge, Tennessee; and Toronto, Canada. Uniform genotype, soil media and planting techniques, treatment procedures, biological measurements, and experimental design were employed. Growth of plants differed among trials as a result of variation in greenhouse environmental conditions according to location and facilities. Larger plants underwent greater absolute but lower relative reductions in biomass after exposure to the higher levels of acidity. A generalized Mitscherlich function was used to model the effects of acidity of simulated rain or dry mass of hypocotyls using data from three laboratories that performed duplicate trials. The remaining data, from three other laboratories that performed only one trial each, were used to test the model. When the laboratory by trial effect was removed (influence of different growth conditions), lack of fit to the Mitscherlich function was insignificant. Thus, a single mathematical model satisfactorily characterized the relationship between acidity and mean plant response. The pH value associated with a 10 percent reduction in mass was 3.3 ± 0.3 for hypocotyls. No value was estimated for shoots because effects on shoots were not significant. The results of this study demonstrate that a generalized exposure-response model can be developed in the presence of large variations in environmental conditions when plant culture and exposure to simulated rain are standardized among laboratories.

Exposure-response functions for effects of acidic rain on vegetation provide a scientific basis for prediction of plant response to different exposure levels.[3] However, differences in response caused by genetic and environmental factors complicate the description of cause and effect relationships. Generalized exposure-response functions, valid for a range of genotypic and phenotypic characteristics, are needed in order to evaluate risk and determine costs and benefits to agriculture from acidic rain.

Thus far, no agreement has been reached on the nature of the exposure-response relationship for effects of acidic rain on vegetation although several models have been investigated.[3,4] Nor have the effects of growth conditions on exposure-response functions been evaluated even though there is reason to believe that environmental conditions change plant response to acidic rain.[6] Disagreement in results between laboratories performing research on acidic rain may be due to many factors[2] and the contribution of different treatment and growing conditions to the divergency of results is not known.

One objective of this investigation was to develop a generalized mathematical model of the relationship between acidity of simulated rain and plant growth. A second objective was to evaluate the effects of environmental factors, such as temperature, light, and humidity, on response to acidity of simulated rain. Exposure of radish plants to simulated acidic rain was repeated in different laboratories with environmental factors allowed to fluctuate according to the conditions occurring in each laboratory's greenhouse at the time the trial was performed. Genotype, cultural techniques, experimental design, procedures for treatment with simulated rain and measurement of effects were standardized. Radish plants were selected as the experimental species because they are known to be susceptible to foliar injury by simulated acidic rain.[1,5,7] They also provide a harvestable product (hypocotyls) in a relatively short time and can be grown in the greenhouse at different times of the year without the use of pesticides. Portions of the data obtained in this study have been published previously.[1,3]

Materials and Methods

Participation and Planning

Representatives from the following laboratories planned the study and supervised its performance: Argonne National Laboratory, Argonne, Illinois (ANL); Boyce Thompson Institute, Ithaca, NY (BTI); Brookhaven National Laboratory,

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Table I. Management and organization of cooperative study.[a]

Planning and organization: ANL, BNL, BTI, CERL, OME, ORNL
Composition of simulated rain solutions: ANL
Analysis of simulated rain solutions: ORNL
Supply of radish seeds: CERL
Analysis of potting media: OME
Procedures and coordination: CERL and BTI
Analysis of data: BTI and CU (Biometrics Unit)
Performance of experiments: ANL, BNL, BTI, OME, ORNL, and OSU

[a] ANL: Argonne National Laboratory; BNL: Brookhaven National Laboratory; BTI: Boyce Thompson Institute; CERL: Corvallis Environmental Research Laboratory; CU: Cornell University; OME: Ontario Ministry of the Environment; ORNL: Oak Ridge National Laboratory; OSU: Oregon State University.

Upton, NY (BNL); Corvallis Environmenntal Research Laboratory, Corvallis, OR (CERL); Oak Ridge National Laboratory, Oak Ridge, TN (ORNL); and Ontario Ministry of the Environment, Toronto, Canada (OME). A written set of guidelines and a form for reporting results were compiled and distributed to all participants. The first trial was performed in the fall of 1981 in greenhouses at each of the six locations with the work at Corvallis performed by the Department of Crop Science at Oregon State University. A second trial was performed by ANL, BTI, and OME between April 1982 and February 1983. Each representative took responsibility for a different aspect of the management and activities of the study (Table I) and for seeing that the guidelines were followed.

Plant Material and Culture

Plant species (*Raphanus sativus* L.), cultivar (Cherry Belle), seed lot, pots, potting media, and planting procedures were standardized among all participants. Pro-Mix B potting mix was rounded to a peak 4 cm above the lip of 10 cm diameter, round, white plastic pots and covered with plastic in order to prevent entrance of simulated rain into the pots. A slit was cut in the center of the plastic and seeds were planted at 2 cm depth. Only 1 seedling was allowed to grow from each pot. For watering, pots were immersed in tap water (see Table II for analysis of tap water) by placement of pots in trays overnight as needed. Temperatures and relative humidities varied among laboratories (Table IV). Supplemental light was used at ANL, BTI, and OME with a 14-hour photoperiod.

Samples of the upper 4 cm of the potting mix were taken from each treatment and sent to the OME laboratory for analysis after completion of the experiment (Table III).

Simulation of Rain

A standardized set of treatment procedures was employed by all participants. Plants were exposed three times per week with one or two days between exposures beginning with the 1st true leaf stage until the plants were four weeks old. Nominal rates of rain deposition were 2 cm per week with 1 hour durations for individual events and median droplet diameters of 0.7 to 1.0 mm. The distance of droplet fall was at least 1.5 m and, if rain was applied intermittently, then the off intervals were to be no greater than 10 minutes. All treatments were given on a single day either in the morning or afternoon. Where insufficient facilities were available for simultaneous exposures at all treatment levels, then the order of treatments was randomized each day of treatment. Plants were placed randomly on greenhouse benches between exposures. Some laboratories repeated the experiment twice while others performed a single trial.

A standardized procedure for preparation of simulated rain solutions was used by all participants based on weighted average concentrations calculated for the New York, Pennsylvania, and Ohio National Atmospheric Deposition Net-

Table II. Nominal and measured pH, sulfate, and nitrate concentrations in simulated rain solutions.

Labs[a]									DW[b]	TW[c]
	Nominal pH									
	5.4	5.0	4.6	4.2	3.8	3.4	3.0	2.6		
	Measured pH									
ANL	4.9	4.8	4.5	4.2	3.8	3.4	3.0	2.7	—	—
BNL	5.5	—	4.5	4.2	—	3.4	3.0	2.6	5.5	—
BTI	5.2	5.0	4.5	4.4	3.8	3.4	3.0	2.6	5.5	7.9
OME	5.4	5.0	4.5	4.2	3.8	3.4	3.0	2.6	5.5	5.4
ORNL	5.8	—	4.7	4.2	—	3.4	3.1	2.6	5.9	—
OSU	4.9	4.7	4.6	4.2	3.7	3.4	3.0	2.6	5.8	7.4
	Nominal sulfate conc, mg/L									
	1	2	2	3	7	15	37	90		
	Measured sulfate conc, mg/L									
ANL	1	2	2	3	6	17	38	79	—	—
BNL	1	—	2	4	—	18	38	95	<0.2	—
BTI	1	2	2	4	7	18	40	95	<0.2	29
OME	1	2	3	3	7	18	40	95	<0.2	<0.2
ORNL	1	—	2	3	—	18	31	92	<0.2	—
OSU	1	1	2	4	8	15	37	92	<0.2	27
	Nominal nitrate conc, mg/L									
	0.5	0.6	0.8	1.4	2.8	6.4	15	38		
	Measured nitrate conc, mg/L									
ANL	0.5	0.6	0.8	1.2	2.7	6.0	15	29		
BNL	0.5	—	0.8	1.4	—	6.2	15	37	<0.01	—
BTI	0.4	0.5	0.8	1.3	2.8	6.1	14	35	<0.01	3.4
OME	0.5	0.6	0.8	1.4	2.9	6.3	16	38	<0.01	<0.01
ORNL	0.5	—	0.7	1.4	—	5.8	10	37	<0.01	—
OSU	0.5	0.5	0.8	1.3	2.8	5.1	13	34	<0.01	12

[a] See Table I for identification of laboratories.
[b] DW = distilled water used to prepare simulated rain solutions.
[c] TW = tap water used for irrigation of plants.

Table III. Environmental conditions in six laboratories during cooperative study.

Lab	Trial	Duration days	No. treatments	Amount of rain, cm	Mean temp. °C	Mean R.H. %
ANL[a]	1	28	10	7.6	26	42
	2	28	10	7.6	23	38
BTI[a]	1	28	10	8.6	21	62
	2	28	10	7.8	24	63
OME[a]	1	29	9	6.7	22	55
	2	29	10	8.0	22	72
BNL	1	29	10	7.6	23	86
ORNL	1	28	10	7.6	29	56
OSU	1	29	9	6.7	18	51

[a] Supplemental lights used with photoperiod of 14 hours.

work sites for 1 June through 30 September 1979. All solutions contained the following background ions (mg/L): ammonium: 0.26, calcium: 0.17, sodium: 0.17, chloride: 0.11, magnesium: 0.04, and potassium: 0.03. Three laboratories used eight treatment levels while the other three laboratories used only four treatment levels. Samples of all simulated rain solutions were collected in plastic containers and mailed to the ORNL laboratory for analysis.

Measurement of Effects and Documentation

Condition of radish foliage was observed and recorded (but not quantified) to determine the characteristics and severity of injury symptoms. Photos were taken using Kodachrome ASA-64 film in sunlight with a Kodak color chart included in the photo to evaluate color rendition.

At harvest, the entire plant was removed from the pot, the below-ground portion rinsed to remove potting mix, and the tops separated from the hypocotyls. Shoots and hypocotyls were dried separately in a forced-draft oven at 60° for at least 48 hours. Weights were taken to the nearest 0.01 g on balances checked against a set of standard weights. Results were reported on standard forms and submitted for tabulation and statistical analysis.

Statistical Methods

Analyses of variance (ANOVA) using programs of SAS[10] were performed on the mean dry weights of 10 radish plants treated simultaneously with simulated acidic rain in experiments that were performed in duplicate trials by three laboratories. The true experimental error was estimated by the error mean square from a randomized complete block design (RCBD) with the laboratory (Lab) by trial combination as the blocking factor and pH as the treatment factor. A further breakdown of the ANOVA was produced by splitting the block sum of squares into Lab, Trial, and Lab × Trial interaction and the error sum of squares into Lab × pH, Trial × pH, and Lab × Trial × pH interactions. The interactions with pH provided a check on the capacity of each laboratory to produce parallel exposure-response relationships at each trial. The Lab × Trial interaction term provided an estimate of the magnitude of differences that existed between trials among laboratories.

A generalized Mitscherlich function, $Y = ai + b0[1 - e^{-b1(pH-b2)}]$[12] was employed to model the response to acidity of simulated rain using nonlinear regression[10] with $ai + b0$ representing the asymptote for each environment (i.e. laboratory trials); $b1$, a dimensionless parameter that controls the slope of the exposure-response function; and $b2$, the theoretical value of pH associated with ai dry weight. When there is only one environment or when all environments are averaged, $ai = 0$, hence $b2$ would be the pH associated with zero dry weight and $b0$ would be the asymptote. The additive effect, ai, does not influence the rate of increase in dry weight which is regulated by the parameter $b1$. Thus, six parallel curves are created for the six environments (trials within laboratories). Removal of the environmental factor would bring all curves to the mean value of the six environments, thus creating a single curve, $y_{adj} = b0^*(1 - e^{-b1^*(pH-b2^*)})$, where y_{adj} equals the dry weight minus the mean dry weight at that environment plus the mean dry weight for all environments.

The Mitscherlich model was explored as an alternative to functions which have been used to model plant response to ozone[8] because the Mitscherlich has natural biological interpretations for its parameters and it has been applied previously to model plant growth response to the supply of nutrients. The Mitscherlich function is intuitively more satisfactory than a plateau-linear model[2] because it does not assume the existence of a sharp break in response. Although the Mitscherlich function is a generalized model that fits many types of data, it would not be appropriate for data that demonstrates a stimulatory effect at low concentrations (quadratic relationship).

The residual sum of squares from the Mitscherlich regression and the error from the RCBD provide a reasonable test for lack of fit.[9] An approximate F statistic for this test is: $F =$ (residual SS − error SS)/($d1 - d2$)/(error SS)/$d2$ where $d1$ and $d2$ are the degrees of freedom associated with the regression residual and sum of squares (SS) from the RCBD respectively.

To avoid complications involved in interpreting unbalanced data, the exposure-response model was developed from results provided by the three laboratories that used all of the same treatment levels. Results from the remaining three laboratories were used to validate the model. A Chi-square goodness-of-fit test was performed using the observed values from the validation data and the predicted values derived from the primary data.

Two procedures were used to provide an estimate of the pH at which hypocotyls begin to decrease in dry mass (threshold value). The first procedure based on the Mitscherlich model estimated the pH associated with 90 percent of the maximum dry weight (pH_{90})[12] after removing the environmental factor. The parameters $b0^*$, $b1^*$, and $b2^*$ in the adjusted model represented the maximum dry weight, rate of increase in dry weight, and the value of pH associated with zero dry weight, respectively. By setting the adjusted dry weight (y_{adj}) equal to $0.9b0^*$ and solving for pH, the value $pH_{90} = b2^* - \ln(0.1)/b1^*$ was obtained. A reparameterization of the Mitscherlich function in terms of pH_{90} resulted in the function $y_{adj} = b0^*[1 - (0.1)e^{-b1^*(pH-pH_{90})}]$. With pH_{90} a parameter in the model, the SAS nonlinear regression program was used to provide an estimate of the asymptotic variance for pH_{90} and a 95 percent confidence interval about the true parameter value.

For the second procedure, a sequential test was performed of ordered alternatives.[13] The only assumption of this procedure is monotonicity, i.e., only decreasing or increasing response among the means, and not a specific exposure-response function.

Table IV. Analysis of potting media in covered pots after exposure to simulated rain.

Measurement	Units	Treatments			
		pH 5.6	pH 4.2	pH 3.4	pH 2.6
pH (in water)	pH	6.7	6.7	6.6	6.5
pH (in $CaCl_2$)	pH	6.3	6.3	6.1	5.9
Ca (in NaCl)	mg/g	9.6	10.3	10.0	10.0
Mg (in NaCl)	mg/g	1.6	1.6	1.8	1.9
K (in NaCl)	mg/g	1.1	1.3	1.3	1.2
CEC[a]	meq/100g	63	68	68	68
SO_4 (in water)	mg/g S	1.4	1.4	1.8	6.3
P (total)	mg/g	1.1	0.9	1.0	0.9
N (total)	mg/g	5.3	5.6	4.7	4.7

[a] CEC: cation exchange capacity.

Results

Analysis of Simulated Rain Solutions

Tap water used for irrigation contained significant amounts of sulfate and nitrate at some locations (Table II). Distilled water used to prepare simulated rain solutions did not contain significant concentrations of these ions. The pH values of solutions were at or close to nominal values (Table II). However, sulfate and nitrate concentrations of simulated rain solutions varied significantly from the nominal values in some laboratories at the higher acidities (pH values of 3.0 and 2.6).

Analysis of Potting Media

The upper 4 cm of potting media generally was quite uniform in chemical composition among treatments with acidic rain with a few exceptions (Table IV). At the pH 2.6 treatment, there appeared to be a slight reduction in pH of the media and a large increase in soluble sulfate. At both pH 3.4 and 2.6, there were small reductions in total nitrogen of the media. Differences among laboratories could not be tested due to the lack of replicate sampling within each laboratory.

Treatment and Environmental Conditions

Some deviations from the planned duration, number of treatments, and amount of rain deposited occurred (Table III). Individual plants were placed randomly on greenhouse benches by two laboratories (ANL and OME), while other laboratories randomized the placement of each treatment group of ten plants. Temperature, humidity, and light intensities (data on light intensity not available) varied considerably among laboratories and trials providing a range of conditions for determining the variation in plant response to simulated acidic rain caused by differences in environmental conditions.

Biomass Measurements

Biomass differed widely among laboratories and trials particularly for hypocotyls (Table V, Figure 1). Hypocotyls were approximately one-third as large at OME, trial 1 than for BTI, trial 2 or ORNL. There were highly significant differences among blocks, more so for hypocotyls than for shoots (Table VI). The significant source of variation contributing to the block effect was the laboratory by trial interaction. Thus, mean radish biomass differed significantly from one trial to another within laboratories.

The mean effect of acidity of simulated rain was signifi-

Table V. Dry mass of radish plants after treatment with simulated acidic rain in six laboratories.

Lab[a]	Trial	Nominal pH of simulated rain							
		5.4	5.0	4.6	4.2	3.8	3.4	3.0	2.6
		Mean mass of shoots, g							
ANL	1	0.57	0.56	0.55	0.56	0.54	0.53	0.54	0.45
	2	0.48	0.54	0.45	0.45	0.47	0.50	0.43	0.39
BTI	1	0.46	0.45	0.55	0.51	0.44	0.47	0.46	0.36
	2	0.44	0.54	0.62	0.60	0.59	0.61	0.54	0.58
OME	1	0.33	0.38	0.35	0.33	0.38	0.38	0.41	0.40
	2	0.73	0.86	0.67	0.71	0.65	0.59	0.45	0.51
BNL	1	0.59	—	0.48	0.54	—	0.48	0.39	0.34
ORNL	1	0.49	—	0.54	0.57	—	0.62	0.50	0.40
OSU	1	0.37	0.47	0.44	0.40	0.35	0.45	0.38	0.36
		Mean mass of hypocotyls, g							
ANL	1	1.15	1.28	1.11	1.11	1.05	0.90	0.83	0.53
	2	0.74	0.77	0.68	0.75	0.73	0.78	0.63	0.35
BTI	1	0.61	0.72	0.71	0.67	0.68	0.63	0.52	0.22
	2	1.33	1.5	1.5	1.37	1.13	1.44	1.27	1.02
OME	1	0.40	0.44	0.44	0.47	0.49	0.40	0.39	0.19
	2	0.97	1.19	1.23	1.19	1.20	1.13	0.83	0.69
BNL	1	0.86	—	0.69	0.77	—	0.70	0.41	0.34
ORNL	1	1.34	—	1.64	1.67	—	1.38	1.16	0.33
OSU	1	0.52	0.55	0.45	0.50	0.44	0.50	0.40	0.33

[a] See Table I for identification of laboratories.

cant for hypocotyls but not for shoots. There were no significant laboratory by pH or trial by pH effects on biomass.

Mitscherlich Exposure-Response Model

A single mathematical model was used to describe the relationship between acidity of simulated rain and biomass after removing the influence of growing conditions (block effects) because there were no significant laboratory by pH or trial by pH effects. The adjusted biomass of hypocotyls at each treatment level produced the exposure-response function depicted in Figure 2. The block by pH interactions from the RCBD gave 0.0076 as an estimate of the true error for hypocotyl dry mass assuming the additive model.

The residual mean squares from the nonlinear regression was 0.0079 for the hypocotyl data (40 d.f.). Lack of fit to the Mitscherlich function was insignificant ($p > 0.1$). The parameter estimates for both the primary and validation data were very similar (Table VII), and the Chi-square goodness-of-fit test was not significant ($p > 0.2$).

The value for pH_{90}, estimated from the Mitscherlich model, was pH 3.3 for hypocotyl dry mass (standard error = 0.14) with a 95 percent confidence interval of pH 3.0 to 3.6. The Williams ordered alternatives test gave an estimated value of 3.4 for the pH at which hypocotyls significantly decreased in dry mass.

Table VI. Analysis of variance for dry mass of radish hypocotyls and shoots after exposure to simulated acidic rain (two trials at each of three laboratories).

Source of Variation	D.F.	Sum of squares	*F* value[a]
		Hypocotyls	
Block	5	4.62	119.8[b]
Lab	2	0.42	0.2
Trial	1	1.50	1.1
Lab × Trial	2	2.70	135.0[b]
pH	7	1.00	18.8[b]
Block × pH	35	0.27	—
Lab × pH	14	0.09	0.6
Trial × pH	7	0.04	0.6
Lab × trial × pH	14	0.14	—
		Shoots	
Block	5	0.37	18.5[b]
Lab	2	0.001	0.004
Trial	1	0.12	1.0
Lab × Trial	2	0.25	25.0[b]
pH	7	0.05	1.8
Block × pH	35	0.14	—
Lab × pH	14	0.05	0.7
Trial × pH	7	0.02	0.6
Lab × Trial × pH	14	0.007	—

[a] *F* statistics for block and pH are calculated using the Block × pH interaction as error, the Lab × Trial interaction is used as error for the Lab and Trial sum of squares, and all other terms are calculated using the Lab × Trial × pH interaction as error.
[b] Significant at the $p < 0.01$ level.

Evaluation of Foliar Symptoms

The highest treatment pH at which traces of necrotic lesions were observed on leaves of radish plants was 3.8 at OME and OSU and 3.4 at ANL, BNL, BTI, and ORNL.

Hypocotyl to Shoot Mass Ratios

Some laboratories, trials and treatments gave mean hypocotyl to shoot ratios as high as 3 (ORNL, pH 4.6 and 4.2; BTI, trial 2, pH 5.4) while, in other situations, mean ratios less than 1 were found (OME, trial 1, pH 3.0 and 2.6; BTI, trial 1, pH 2.6; ORNL, pH 2.6; ANL, pH 2.6; OSU, pH 2.6). More

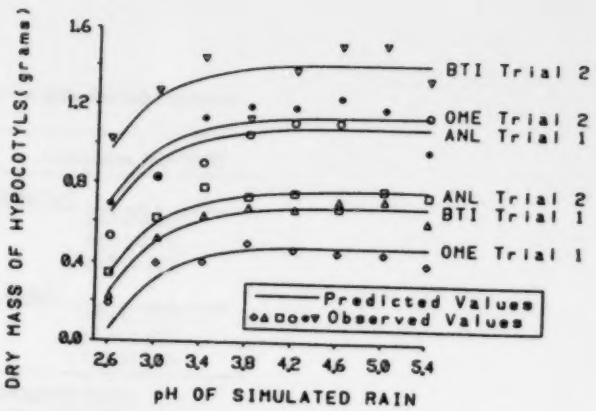

Figure 1. Individual exposure-response relationships between the pH of simulated rain and hypocotyl mass of radish for two trials performed in each of three laboratories. Solid lines were calculated from the Mitscherlich model.

pronounced reductions in the mean hypocotyl/shoot mass ratio occurred at the higher acidities in every laboratory and trial with the exception of OME, trial 2. For some laboratories and trials, the ratio at pH 2.6 was less than half of the ratio at pH 5.4 (OME, trial 1; ORNL; BTI, trial 1).

Discussion

There are several unique contributions of this study: 1) standardized methods and procedures were employed among a group of laboratories to evaluate the response of a single plant species to simulated acidic rain; 2) quality control procedures were used to check on chemistry of simulated rain and penetration of simulated rain into potting media; 3) the Mitscherlich function, not previously applied to model plant response to pollutants, was used to obtain a generalized exposure-response relationship for effects of acidic rain

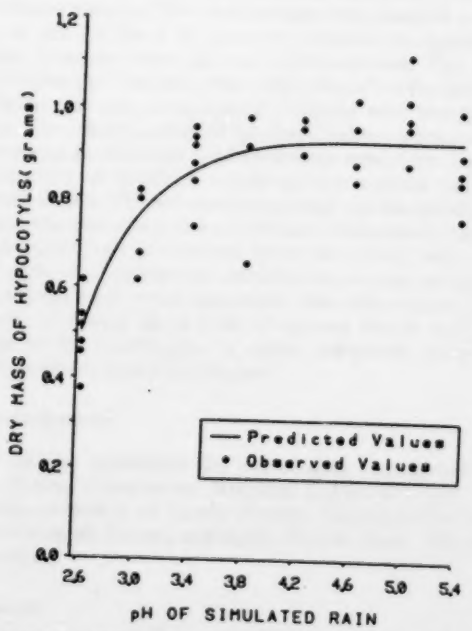

Figure 2. Generalized exposure-response relationship between the pH of simulated rain and hypocotyl mass of radish. Solid line was calculated from the Mitscherlich model after removing the influence of environmental conditions.

Table VII. Parameter estimates for the Mitscherlich exposure-response model.

Parameter	Primary data (S.E.)	Validation data (S.E)
	Hypocotyl	
b0*[a]	0.92 (0.019)	1.17 (0.091)
b1*[b]	2.13 (0.460)	2.18 (1.012)
b2*[c]	2.25 (0.090)	2.45 (0.112)
	Shoot	
b0*	0.52 (0.006)	0.54 (0.023)
b1*	3.74 (1.56)	2.38 (1.413)
b2*	2.19 (0.177)	2.13 (0.298)

[a] b0*: asymptote after removing environmental factor.
[b] b1*: rate of increase of biomass with increasing pH.
[c] b2*: theoretical point of intersection with × axis after removing environmental factor.

on plant biomass; and 4) an estimate was made of the threshold of acidity for reductions in biomass with a set of data that included a range of environmental conditions and plant growth rates. The results demonstrate that, by standardizing methods and procedures, it is possible to establish a generalized model of exposure-response model as long as the qualitative characteristics of plant response to acidic rain are unaffected by the different environmental conditions.

The exposure-response function developed from this set of experiments, repeated at different locations and times, provides a more reliable basis for predictive purposes because many laboratories contributed to its development and verification and because it was obtained over a range of environmental conditions. The use of greenhouses facilitated the development of the model because resources already were available, the cost was moderate, and the experiment could be repeated within the course of a year. Models developed with greenhouse-grown plants need to be tested in the field to determine their usefulness and relevance to actual cultural, climatic, and edaphic conditions for particular crops and regions of the country. It is particularly important to know whether other factors relating to rainfall occurrence, such as fluctuations in rainfall fequency, intensity and composition with time and duration of dry intervals,[5] affect exposure-response relationships. Standardized experiments that are performed with a range of genotypes also would incorporate genetic variation within the exposure-response model. If the form as well as the magnitude of plant response to acidity of rain is changed by genotype or rainfall conditions, then qualitatively different exposure-response functions would have to be developed for individual species or groups of species.

The main source of variation in this study was among trials within laboratories and not just from one laboratory to another because climatic conditions change with time. The repetition of trials within laboratories made it possible to evaluate the variation of response among laboratories and to develop a generalized model. Growth conditions vary with time (season) in greenhouses perhaps more than in the field where most crops are planted only during a particular time of the year.

There were important similarities and differences in results among trials. Similarities included the decrease in biomass with higher concentrations of acidity of simulated rain, greater effects on below-ground than above-ground biomass, the first appearance of foliar symptoms at pH 3.4 to 3.8, and the close fit of all results to the Mitscherlich model. Differences included the different rates of growth due to different environmental conditions, the different ratio of above- to below-ground biomass in different trials where conditions produced plants of different size, and the greater absolute reductions in biomass due to acidity under conditions that produced larger plants.

The Mitscherlich function may be particularly attractive to biological scientists and to policy analysts for describing the response of plants to pollutants. The parameters of the adjusted model, namely the influence of environmental conditions (a constant), the severity of injury (slope), and the no-effect region (asymptote) have biological relevance. An alternative model, the Weibull function,[8] was compared with the Mitscherlich and it does equally well at describing plant response to acidity and provides similar results for the estimated pH_{50}. Both models provide a way of interpreting the consequences of changes in levels of acidity of precipitation for plant growth. The Mitscherlich model, however, appeared to be more flexible within the "threshold" range (i.e. as the function begins to curve away from the asymptote) and more adaptable due to the ease of selecting starting values for the nonlinear estimation procedure.

The two methods used for calculating threshold pH values gave similar results. The model-dependent method gave a value of pH 3.3 for a 10 percent reduction in hypocotyl biomass averaged over different environments. The non-model dependent method gave a pH value of 3.4 for the first detectable decrease in hypocotyl biomass with increasing acidity. The identification of threshold values is dependent on genotype, environment and treatment conditions and on the definition of threshold values and the method used for their calculation. The nature of the model and its underlying assumptions also affect the calculation of threshold values. The threshold values obtained from this study, while obtained under conditions that simulate only certain aspects of ambient exposures, are in agreement with other reports that the level of acidity in rainfall of eastern North America appears to be insufficient to cause reductions in plant growth by direct injury to foliage.[3,6]

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